

EFFECT OF SODIUM NITRITE CONCENTRATION ON N-NITROSODIMETHYLAMINE FORMATION IN FRANKFURTERS

INTRODUCTION

UNTIL 1926 nitrate salts were used in the United States in the fixing of color of meat products by the "corning" or curing process. Haldane (1901) demonstrated that this function takes place through the reduction of nitrate to nitrite. As a result of the work of Kerr et al. (1926), the Department of Agriculture permitted the use of sodium nitrite in meat curing. In addition to color fixation, nitrite serves to develop the characteristic flavor of the cured meat products (Brooks et al., 1940; Cho and Bratzler, 1970) and preserve them against bacterial spoilage (Silliker et al., 1958). In general cured meat products have enjoyed a long period of safe consumption. Concern over the use of food additives as potential public health hazards has increased in recent years. With it, the use of sodium nitrite has been questioned. One of the main reasons for this is that sodium nitrite, under acidic conditions, reacts with many amines, particularly secondary amines to form N-nitrosamines which may be tumorigenic or carcinogenic (Druckery et al., 1967).

The presence in meat products of free amines or amine precursors such as proteins, amino acids, phospholipids and other compounds poses a situation in which they may possibly react with nitrite to form N-nitrosamines. There have been reports of finding a nitrosamine in cured meat products (Möhler and Mayrhofer, 1968; Ender and Ceh, 1968; Freimuth and Gläser, 1970). The methods employed may have lacked the necessary sensitivity and selectivity to give positive identification. Other workers (Fazio et al., 1971; Fiddler et al., 1971; Telling et al., 1971) have not confirmed the presence of nitrosamines in various cured meat products at a level greater than 25 ppb. However, recently the presence of dimethylnitrosamine (DMNA) in three frankfurter samples, one of which contained 80 ppb, has been demonstrated (Wasserman et al., 1972). Studies to date indicate that DMNA occurrence is random and no adequate explanation is available. This could be due to a number of variables which are involved in the preparation of cured meat products e.g., the age and condition of the meat, the concentration of nitrite and the type and amounts of other ingredients used, the

actual processing conditions, and subsequent time and temperature of storage. In view of the lack of information available on the effect of these processing factors on nitrosamine formation, a study was undertaken to determine the effect of nitrite concentration on DMNA formation in frankfurters containing sugar and salt, but without nitrate, ascorbate and delta-gluconolactone. The latter three ingredients are sometimes used commercially.

EXPERIMENTAL

Frankfurter preparation

Fresh beef and pork were trimmed to desired fat levels, 5 and 17% respectively, and ground through a 3/4-in. plate. The beef, pork and pork fat were stored under vacuum in Cryovac bags at 0°F. The day prior to processing, the frozen portions were removed from the freezer and allowed to thaw. The meat was ground through a 5/8-in. plate, then a 3/16-in. plate. A formulation based on the results of analyses of raw materials was used to produce frankfurters containing 9–10% added moisture and 30% fat. The weights (kg) of the components of the emulsion were: lean beef—3.934; lean pork—2.514; pork fat—3.552; ice—2.287; salt—0.251; and sugar—0.198. Nitrate, ascorbate and delta-gluconolactone were omitted from the formulation to reduce the number of variables that may effect nitrosamine formation. The ingredients were comminuted in a high-speed Stephan Universal Schnellkuter Type USF25 chopper equipped with a thermocouple and a sealable Plexiglass plate. Under 110 mm Hg vacuum, the initial temperature of the mixture was about 35°F. The chopping was continued up to a temperature of 60°F, taking approximately 5 min. The amount of sodium

nitrite required was weighed, dissolved in 15 ml distilled water, and mixed thoroughly with 1.274 kg emulsion (equivalent to 1 kg of meat) using a Hobart Model N-50 mixer, to give the following levels of sodium nitrite added in ppm with respect to meat (lean plus fat): 150, 750, 1050, 1500 and 2500.

The emulsion was then stuffed into size 23 Nojax frankfurter casings using a screw-type stainless steel sausage stuffer having a capacity of 500g. The franks were linked using a Model MF Ty Linking Machine and cooked in a Dry-Sys Smokehouse using a 90-min commercial program of increasing heat and controlled humidity: 1/2 hr dry bulb (DB) 130°F, wet bulb (WB) 0; 1/2 hr DB 150°F, WB 130°F; 1/2 hr DB 170°F, WB 150°F. A light smoke was generated in a Mepaco apparatus and introduced into the smokehouse for the entire period. After the franks had reached an internal temperature of 160°F (about 2 hr), one batch containing different levels of added sodium nitrite was removed and the remainder was heated and smoked for an additional 2 hr (DB 170°F, WB 150°F). These frankfurters had an average internal temperature of 160°F. After removal from the smokehouse, the frankfurters were immersed in an ice-water bath for 3 min, dried, weighed and stored overnight at 36°F. A sample of the franks processed normally for approximately 2 hr at the 150 ppm level was analyzed according to AOAC procedures and found to contain 53.40% moisture, 29.42% fat and 14.42% protein. The remaining franks were then vacuum packaged, frozen and kept in a freezer at 5°F until analyses were carried out.

When needed, the frankfurters containing different levels of sodium nitrite were thawed, ground, mixed and an aliquot taken for analyses. The residual nitrite was determined by the AOAC method; the average loss in sodium nitrite in the 2-hr and 4-hr processed frankfurters was 48.6% and 55.4% respectively.

Table 1—The effect of sodium nitrite concentration on dimethylnitrosamine formation in frankfurters

NaNO ₂ added mg/kg meat	Processing time			
	2 Hr		4 Hr	
	NaNO ₂ residual mg/kg	DMNA μg/kg ^a	NaNO ₂ residual mg/kg	DMNA μg/kg ^a
150	67	trace	53	trace
750	361	3	310	8
1050	574	8	473	12
1500	811	10	724	14
2500	1386	19 MS	1345	19 MS

^aCorrected using recovery of sample with 20 μg/kg added; MS—confirmed by mass spectrometry.

Analytical procedures

The methodology employed for the determination of DMNA in frankfurters is a modification of the procedure described by Howard et al. (1970). The method is as follows: the ground frankfurter samples are subjected to digestion with methanolic potassium hydroxide, followed by distillation from aqueous alkaline solution. The distillate is extracted with methylene chloride. The combined extracts are washed with base and dried over anhydrous sodium sulfate, concentrated and subjected to column chromatography using Florisil acidified with hydrochloric acid. After the column is washed with hexane, the sample is eluted with methylene chloride, concentrated and the DMNA present determined by GLC. The average recovery of DMNA in an aliquot of the same sample with 20 ppb added was 76%.

Gas-liquid chromatography

Varian Aerograph Model 1740-1 gas chromatograph equipped with two 9 ft x 1/8 in. OD stainless steel columns packed with 15% Carbowax 20M-TPA on 60-80 Gas-Chrom P, was conditioned overnight at 180°C, and installed for on-column injection. The standard flame ionization detector was modified for use as an alkali flame ionization detector by the use of a potassium chloride coated coil as described by Howard et al. (1970). The flow conditions used were: helium 58, hydrogen 45 and air 188 ml/min. Helium flow was monitored continuously with Matheson Model LF 100 mass flowmeter. Hydrogen flow and, to a lesser extent, air flow were adjusted slightly from time to time in order to maintain desired detector sensitivity. Electrometer range used was 10^{-12} amp/mv. Injector port and detector temperatures were 190 and 250°C, respectively. Column temperature was 115° isothermal for routine analyses involving only DMNA.

GLC-mass spectrometric analysis

The analytical procedure described above, which used 25g of frankfurter, was repeated as needed to provide sufficient DMNA for confirmation of identity by mass spectrometry. An identical gas-liquid chromatograph equipped with a flame ionization detector and column was used as for the determination of DMNA. A column temperature of 115°C was used with injector port and detector temperatures of 200° and 230°, respectively. Helium, at a flow rate of 25 ml/min, was used as the carrier gas. The hydrogen and air flow rates were 40 and 335 ml/min., respectively.

The column effluent was split approximately 1:1 and passed into a DuPont Model 21-492 mass spectrometer equipped with a jet-type separator via an inlet line heated at 200°C. The mass spectra were obtained at an ionizing voltage of 70 eV and an ion source temperature of 200°C using a CEC model 5-124A recording oscillograph with a linear scan rate of 20 sec/decade.

RESULTS & DISCUSSION

THE PROCESSING study was performed three times under the same 2- and 4-hr processing conditions covering a wide range of sodium nitrite concentrations added to frankfurter emulsion. Analyses of the frankfurters for DMNA yielded similar values for all of the studies. Representative data from one of these experiments is shown in Table 1.

Ideally the identification of DMNA in natural products should be unambiguous. Where very small amounts of DMNA are encountered, confirmation may be accomplished by mass spectrometry. In the present study, however, DMNA concentrations of less than 10 µg/kg in frankfurter samples prepared as described were insufficient for confirmation. Treating larger amounts of frankfurter and/or concentrating multiple extracts to obtain sufficient material resulted in the presence of artifacts which interfered with mass spectral interpretation. Therefore, a minimum level of DMNA 10 µg/kg was considered significant. When DMNA was not characterized by mass spectrometry, identity was based only on its GLC retention time. In the discussion of the results, the term "apparent" DMNA is used to indicate levels at which mass spectral confirmation could not be made.

The U.S. Code of Federal Regulations (1971) limits the quantity of sodium nitrite that may be added to comminuted meat products, including frankfurters, to 156 mg/kg (1/4 oz per 100 lb chopped meat). At levels of up to about five times this permissible concentration no significant amount of DMNA was observed when the normal 2-hr processing procedure was used; however, a DMNA concentration bordering on significance, i.e., 8 µg/kg meat, was obtained on heating an additional 2 hr. Concentrations of apparent or confirmed DMNA of 10 µg/kg and greater were found in frankfurters made with levels of sodium nitrite of 1500 mg/kg or higher, regardless of processing time. In addition to 2500 mg sodium nitrite per kg meat, levels of 3075 and 4250 mg/kg were also studied in another series of the same experiment, and concentrations of DMNA up to 60 µg/kg were found for the 4-hr processed frankfurters and confirmed readily by mass spectrometry. Sodium nitrite concentrations between 150 and 750 ppm sodium nitrite, for both the 2- and 4-hr processing times, gave negligible amounts of DMNA.

For most of the levels of added nitrite at which apparent or confirmed DMNA could be demonstrated, there was an increase in DMNA concentration when the frankfurters were treated an additional 2 hr. The extra heating period is not normally used commercially; however, its use here represents an extreme in experimental conditions.

Residual nitrite analyses showed that about one-half of the sodium nitrite added was still present after processing. Commercially prepared frankfurters made with 156 mg/kg sodium nitrite usually have residual nitrite levels ranging from 10-30% of the added material. In previous studies, we have observed that more nitrite is lost when processing in combination with ascorbate and nitrate, and the

concentration of nitrite continues to decrease upon storage. Therefore the significance of the higher values found in this study cannot be evaluated at this time.

CONCLUSIONS

FRANKFURTERS conforming to U.S. Federal Regulations contain a concentration of sodium nitrite insufficient to produce, under the processing conditions described in this paper, amounts of DMNA measurable by our procedures. However, under laboratory conditions DMNA can be formed in a meat product. Concentrations of sodium nitrite up to about ten times the legal limit may lead to significant nitrosamine formation. This study does not explain why DMNA can be isolated from some commercial cured meat products containing the permissible level of sodium nitrite. Many variables have been suggested or can be conceived as contributing to DMNA formation in the products, e.g., localized high concentrations of nitrite in emulsions due to inadequate homogenization during processing, the effect of other cure ingredients, storage conditions, etc. Further work is needed to establish the contributions, if any, of these variables.

REFERENCES

- Brooks, J., Haines, R.B., Moran, T. and Pace, 1940. The function of nitrate, nitrite and bacteria in the curing of bacon and hams. Dept. Sci. & Indust. Res., Food Invest. Spec. Rept. No. 49, (Great Britain).
- Cho, I.C. and Bratzler, L.J. 1970. Effect of sodium nitrite on flavor of cured pork. J. Food Sci. 35: 668.
- Code of Federal Regulations. 1971. Animal and Animal Products, Chapter III Section 318.7, Rev Jan. 1, Title 9.
- Druckrey, H., Preussmann, R., Ivankovic, S., Schmal, D., Afkham, J., Blum, G., Memmel, H.D., Muller, M., Petropoulos, P. and Schneider, H. 1967. Organotropic carcinogenic effects of 65 different N-nitroso compounds on BD rats. Z. Krebsforsch. 69: 103.
- Ender, F. and Ceh, L. 1968. Occurrence of nitrosamines in foodstuffs for human and animal consumption. Food Cosmet. Toxicol. 6: 569.
- Fazio, T., White, R.H. and Howard, J.W. 1971. Analysis of nitrite- and/or nitrate-processed meats for N-nitrosodimethylamine. J. Assoc. Offic. Anal. Chem. 54: 1157.
- Fazio, T., Damico, J.N., Howard, J.W., White, R.H. and Watts, J.O. 1971. Gas chromatographic determination and mass spectrometric confirmation of N-nitrosodimethylamine in smoke-processed marine fish. J. Agr. Food Chem. 19: 250.
- Fiddler, W., Doerr, R.C., Ertel, J.R. and Wasserman, A.E. 1971. Gas-liquid chromatographic determination of N-nitrosodimethylamine in ham. J. Assoc. Offic. Anal. Chem. 54: 1160.
- Freimuth, U. and Gläser, E. 1970. The occurrence of nitrosamines in foods. Die Nahrung 14: 357.
- Haldane, J. 1901. The red colour of salted meat. J. Hyg. 1: 115.
- Howard, J.W., Fazio, T. and Watts, J.O. 1970. Extraction and gas chromatographic determination of N-nitrosodimethylamine in smoked fish. Application to smoked nitrite-treated chub. J. Assoc. Offic. Anal. Chem. 53: 269.
- Kerr, R.H., Marsh, C.T.N., Schroeder, W.F. and Boyer, E.A. 1926. The use of sodium nitrite in the curing of meat. J. Agr. Res. 33: 541.
- Möhler, K. and Mayrhofer, O.L. 1968. Detec-

tion and determination of nitrosamines in foodstuffs. Z. Lebensm.-Unters. Forsch. 135: 313.

Illiker, J.H., Greenberg, R.A. and Schack, W.R. 1958. Effect of individual curing ingredients on the shelf-stability of canned comminuted meats. Food Technol. 12: 551.

Welling, G.M., Bryce, T.A. and Althorpe, J. 1971. Use of vacuum distillation and gas

chromatography-mass spectrometry for determination of low levels of volatile nitrosamines in meat products. J. Agr. Food Chem. 19: 937.

Wasserman, A.E., Fiddler, W. and Kushnir, I. 1972. Unpublished results.

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Reference to brand names or firm name does not constitute endorsement by the U.S. Dept. of Agric. over others of a similar nature not mentioned.